Manual
PHERO-sub E series

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Revision: 1.0

Instruction Manual

PHEROsub E Series

Horizontal Agarose Gel

Electrophoresis Systems

Catalogue Numbers

111100-001 111100-002 111100-003	PHERO-sub 0710-E	111210-001	PHERO-sub 1008-E
111300-001 111300-002 111300-003	PHERO-sub 1010-E	111400-001 111400-002 111400-003 111400-004	PHERO-sub 1515-E
111500-001 111500-002 111500-003	PHERO-sub 2020-E	111600-001 111600-002 111600-003 111600-004	PHERO-sub 2632-E

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Contents

SAFETY PRECAUTION..... 3
PACKING LISTS:..... 4
PHERO-sub 0710-E..... 4
PHERO-sub 1010-E..... 4
PHERO-sub 1515-E..... 5
PHERO-sub 2020-E..... 5
PHERO-sub 2632-E..... 6
PHERO-sub 1008-E..... 6
Usage Guidance and restrictions:..... 7
Care and Maintenance:-..... 8
Setting up the Horizontal Gel Tanks:-..... 9
Gel Preparation:-..... 10
Gel Pouring:-..... 11
Using trays with Casting Dams (Note not PHERO-sub 2632-E):-..... 11
Using The Flexicaster (Note not PHERO-sub 2632-E) :-..... 11
Using Traditional tape method:-..... 12
Casting in the Mini Fast Unit:-..... 13
Running the Gel:-..... 14
Gel Staining and Viewing:-..... 15
References..... 15
Combs – MC Denotes Multi Channel Pipette compatible..... 17
Warranty..... 20

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SAFETY PRECAUTION

WHEN USED CORRECTLY, THESE UNITS POSE NO HEALTH RISK. HOWEVER, THESE UNITS CAN DELIVER DANGEROUS LEVELS OF ELECTRICITY AND ARE TO BE OPERATED ONLY BY QUALIFIED PERSONNEL FOLLOWING THE GUIDELINES LAID OUT IN THIS INSTRUCTION MANUAL.

ANYONE INTENDING TO USE THIS EQUIPMENT SHOULD READ THE COMPLETE MANUAL THOROUGHLY.

THE UNIT MUST NEVER BE USED WITHOUT THE SAFETY LID CORRECTLY IN POSITION.
THE UNIT SHOULD NOT BE USED IF THERE IS ANY SIGN OF DAMAGE TO THE EXTERNAL TANK OR LID.

THESE UNITS COMPLY WITH THE STATUTORY CE SAFETY DIRECTIVES:
73/23/EEC: LOW VOLTAGE DIRECTIVE: IEC 1010-1:1990 plus AMENDMENT 1:1992
EN 61010-1:1993/BS EN 61010-1:1993

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PACKING LISTS:

PHERO-sub 0710-E

111100-001, 111100-002, 111100-003

Units include tank, lid and electrodes and include the following accessories:-

	Tray	Tray Dams	Combs	Loading Guides	Cables
111100-001	111130-001 7 x 7cm (W x L)	111140-002 Pack of 2	111121-004 1mm, 8 sample	111140-003 – Strips 111140-004 – Platform	111110-006
111100-002	111131-001 7 x 10cm (W x L)	111140-002 Pack of 2	2 x 111121-004 1mm, 8 sample	111140-003 – Strips 111130-004 – Platform	111110-006
111100-003	111130-001 111131-001	111140-002 Pack of 2	2 x 111121-004 1mm, 8 sample	111140-003 – Strips 111140-004 – Platform	111110-006

PHERO-sub 1010-E

111130-001, 111130-002, 111130-003

Units include tank, lid and electrodes and include the following accessories:-

	Tray	Tray Dams	Combs	Loading Guides	Cables
111130-001	111330-001 10 x 7cm (W x L)	111340-001 Pack of 2	2 x 111321-007 1mm, 16 sample	111340-003 – Strips 111340-004 – Platform	111310-006
111130-002	111331-001 10 x 10cm (W x L)	111340-001 Pack of 2	2 x 111321-007 1mm, 16 sample	111340-003 – Strips 111340-004 – Platform	111310-006
111130-003	111330-001 111331-001	111340-001 Pack of 2	2 x 111321-007 1mm, 16 sample	111340-003 – Strips 111340-004 – Platform	111310-006

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PHERO-sub 1515-E

111400-001, 111400-002, 111400-003, 111400-004

Units include tank, lid and electrodes and include the following accessories:-

	Tray	Tray Dams	Combs	Loading Guides	Cables
111400-001	111430-001 15 x 7cm (W x L)	111440-002 Pack of 2	2 x 111421-007 1mm, 20 sample	111440-003 – Strips 111440-004 – Platform	111410-006
111400-002	111431-001 15 x 10cm (W x L)	111440-002 Pack of 2	2 x 111421-007 1mm, 20 sample	111440-003 – Strips 111440-004 – Platform	111410-006
111400-003	111432-001 15 x 15cm (W x L)	111440-002 Pack of 2	2 x 111421-007 1mm, 20 sample	111440-003 – Strips 111440-004 – Platform	111410-006
111400-004	111430-001 111431-001 111432-001	111440-002 Pack of 2	2 x 111421-007 1mm, 20 sample	111440-003 – Strips 111440-004 – Platform	111410-006

PHERO-sub 2020-E

111500-001, 111500-002, 111500-003

Units include tank, lid and electrodes and include the following accessories:-

	Tray	Tray Dams	Combs	Loading Guides	Cables
111500-001	111530-001 20 x 10cm (W x L)	111540-002 Pack of 2	2 x 111521-006 1mm, 20 sample	111540-003 – Strips 111540-004 – Platform	111510-006
111500-002	111531-001 20 x 20cm (W x L)	111540-002 Pack of 2	2 x 111521-006 1mm, 20 sample	111540-003 – Strips 111540-004 – Platform	111510-006
111500-003	111530-001 111531-001	111540-002 Pack of 2	2 x 111521-006 1mm, 20 sample	111540-003 – Strips 111540-004 – Platform	111510-006

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PHERO-sub 2632-E

111600-001, 111600-002, 111600-003, 111600-004

Units include tank, lid and electrodes and include the following accessories:-

	Tray	Combs	Loading Guides	Cables
111600-001	111630-001 26 x 16cm (W x L)	6 x 111621-001 1mm, 28 sample	111640-003 – Strips 111640-004 – Platform	111610-006
111600-002	111631-001 26 x 24cm (W x L)	6 x 111621-001 1mm, 28 sample	111640-003 – Strips 111640-004 – Platform	111610-006
111600-003	111632-001 26 x 32cm (W x L)	6 x 111621-001 1mm, 28 sample	111640-003 – Strips 111640-004 – Platform	111610-006
111600-004	111630-001 111631-001 111632-001	6 x 111621-001 1mm, 28 sample	111640-003 – Strips 111640-004 – Platform	111610-006

PHERO-sub 1008-E

Unit includes tank, lid and electrodes and the following accessories:-

	Casting Gates	Combs	Cables
111200-001	111240-002 Pack of 2	2 x 111221-003 1.5mm, 8 sample	111210-006

The packing lists should be referred to as soon as the units are received to ensure that all components have been included. The unit should be checked for damage when received.

Please contact your supplier if there are any problems or missing items.

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Usage Guidance and restrictions:

• Maximum altitude 2,000m.
• Temperature range between 4°C and 65°C.
• Maximum relative humidity 80% for temperatures up to 31^{O}C decreasing linearly to 50% relative humidity at 40^{O}C.
• Not for outdoor Use.

This apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664. POLLUTION DEGREE 2, states that: “Normally only non-conductive pollution occurs. Occasionally, however, a temporary conductivity caused by condensation must be expected”.

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Care and Maintenance:-

Cleaning Horizontal Units

Units are best cleaned using warm water and a mild detergent. **Water at temperatures above 60^0 C can cause damage to the unit and components.**

The tank should be thoroughly rinsed with warm water or distilled water to prevent build up of salts but care should be taken not to damage the enclosed electrode and vigorous cleaning is not necessary or advised.

Air drying is preferably before use.

The units should only be cleaned with the following:-

Warm water with a mild concentration of soap or other mild detergent.

Compatible detergents include dishwashing liquid, Hexane and Aliphatic hydrocarbons

The units should not be left to in detergents for more than 30 minutes.

The units should never come into contact with the following cleaning agents, these will cause irreversible and accumulative damage:-

Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol

Alkalis.

Rnase Decontamination

This can be performed using the following protocol:-

Clean the units with a mild detergent as described above.

Wash with 3% hydrogen peroxide (H2O2) for 10 minutes.

Rinsed with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water,

Caution: DEPC is a suspected carcinogen. Always take the necessary precautions when using.

RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

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Setting up the Horizontal Gel Tanks:-

Instructions for fitting Electrode Cables.

1. Note the position of the lid on the unit. This shows the correct polarity and the correct orientation of the cables, black is negative and red positive.
2. Remove the lid from the unit. Note if the lid is not removed, fitting the cables may result in un-tightening of the gold plug and damage to the electrode.
3. Screw the cables into the tapped holes as fully as possible so that there is no gap between the lid and the leading edge of the cable fitting.
4. Refit the lid.

Instructions for fitting Loading Guides (Not FMMS10).

These can be fitted to enhance visibility of the wells if desired. They can be fitted to the white vinyl platform sheet or to the unit itself.

1. Seat the tray in the unit and note the position of the comb grooves. The samples run black to red but the trays can be used frontward or backwards so ensure that the comb grooves closest to the black electrode are marked.
2. Remove the tray.
3. Peel the back off of the loading guide and carefully apply the loading guide directly to the gel platform.

The unit is now ready to be used.

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Gel Preparation:-

1. Table 1 below shows the volume of agarose solution required to make the desired agarose gel for each unit tray size. For a standard 0.7% agarose gel, add 0.7 grammes of agarose to 100 ml of 1x TAE or TBE solution. The same 1 x solution should be used in the tank buffer solution.

Table 1.

PHERO-sub 0710-E		PHERO-sub 1010-E		PHERO-sub 1515-E		PHERO-sub 2020-E	
Tray	**Gel volume for a 0.5mm thick gel**	**Tray**	**Gel volume for a 0.5mm thick gel**	**Tray**	**Gel volume for a 0.5mm thick gel**	**Tray**	**Gel volume for a 0.5mm thick gel**
7 x 7cm	25ml	**10 x 7cm**	35ml	**15 x 7cm**	52.5ml	**20 x 10cm**	100ml
7 x10cm	35ml	**10 x 10cm**	50ml	**15 x 10cm**	75ml	**20 x 20cm**	200ml
				15 x 15cm	112.5ml		

PHERO-sub 2632-E		PHERO-sub 1008-E	
Tray	**Gel volume for a 0.5mm thick gel**		**Gel volume for a 0.5mm thick gel**
26 x 16cm	208ml		40ml
26 x 24cm	312ml		
26 x 32cm	416ml		

2. Add the agarose powder to a conical flask.

3. Add the appropriate amount of 1x TAE or TBE solution from the table above. To prevent evaporation during the dissolving steps below, the conical flask should be covered with parafilm.

4. Dissolve the agarose powder by heating the agarose either on a magnetic hot plate with stirring bar or in a microwave oven. If using the microwave method, the microwave should be set at around a 400 watt or medium setting and the flask swirled every minute. The solution should be heated until all crystals are dissolved. This is best viewed against a light background. Crystals appear as translucent crystals. These will interfere with sample migration if not completely dissolved.

The gel must be cooled to between 50°C and 60°C degrees before pouring.

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Gel Pouring:-

The multiSUB range of units allow three different methods of gel casting:-

Casting Dams

Flexicaster

Traditional Tape

Using trays with Casting Dams (Note not PHERO-sub 2632-E):-

1. Fit the casting dams over each end of the tray and place onto a level surface. The dams should be fitted so that there is no gap between the sides of the tray and the groove in the dams. This will ensure that there is no possibility of gel leakage.
2. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
3. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
4. Allow the agarose to set, ensuring that the gel remains undisturbed.
5. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

Using The Flexicaster (Note not PHERO-sub 2632-E) :-

1. Level the Flexicaster base by adjusting the feet so that the bubble is exactly central.
2. Insert the desired length tray into the Flexicaster such that one end of the tray is pushed up and seals against the silicone mat of the permanent end of the Flexicaster.
3. Position the movable end of the Flexicaster so that the silicone mat is pushed against the other end of the tray.

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4. Turn the cam so that the silicone mat tightly seals against the side of the tray. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
5. Allow the agarose to set, ensuring that the gel remains undisturbed.
6. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

Using Traditional tape method:-

1. Autoclave or plastic backed general tape should be used. A length 5cm longer than the width of each end of the tray should be cut. One length should be placed over one end of the tray and stuck m1cm in from the tray edge. This should then be folded and the edges sealed securely. Repeat for the other end and place onto a level surface for gel pouring.
2. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
3. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
4. Allow the agarose to set, ensuring that the gel remains undisturbed.
5. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

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Casting in the Mini Fast Unit:-

1. Fit the casting gates into the grooves in the unit which are 10mm from the platinum wire. Ensure that these are seated as deeply as possible. This will ensure that a good seal is formed and that there is no possibility of gel leakage. A small amount of Vaseline or sealing grease on the bottom and side edges of the gates will give further leakage protection.
2. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
3. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
4. Allow the agarose to set, ensuring that the gel remains undisturbed.
5. Carefully remove the gel casting gates and comb.

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Running the Gel:-

1. Mix the sample to be loaded with sample buffer – see solutions for common sample buffers. Usually 3ul of sample buffer is adequate but less may be used with sample volumes of less than 10ul.
2. Fill the unit with buffer until the gel is just flooded with buffer. This will give the fastest resolution times. For enhanced quality of resolution of sample, fill the unit to 5mm above the gel.
3. Load the samples into the wells using pipettes. Multi-channel pipettes can be used for
loading samples with MC compatible combs, see listing in accessories for identification of these.
4. Carefully place the lid on the tank and connect to a power supply.
5. Typically gels are run at between 90 and 150 volts. However, maximum voltages are indicated on the serial badge of each unit. It should be noted that higher voltages generally give faster but poorer quality sample resolution.

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Gel Staining and Viewing:-

The Multi Sub trays and the Mini Fast unit allow staining to be performed without removing the gel from the tray (unit for Fast Mini) if this is preferred.

1. Transfer the gel to a vessel containing the appropriate volume of 0.5 µg/ml ethidium bromide stain for 15–30 minutes, see solutions for stock stain concentration and adjust to the volume used accordingly. The entire gel should be covered.

NOTE:- Ethidium bromide is a suspected carcinogen and the necessary safety precautions should be undertaken.

2. De-stain the gel for 10–30 minutes in distilled water again ensuring the gel is completely immersed.
3. Rinse the gel twice for a couple of seconds with distilled water.
4. Transfer the gel to a UV Transilluminator.
5. The samples will often appear as brighter, clearer bands when photographed or viewed using a gel documentation system. However if the gel bands are too faint then the staining procedure should be adjusted so that there is less de-staining. If there is too much background then the staining procedure should be adjusted so that there is more de-staining.

References

1. Sambrook, Fritsch, and Maniatis, **Molecular Cloning A Laboratory Manual,** Second Edition,
Cold Spring Harbor Laboratory Press, 1989.

2. **Current Protocols in Molecular Biology,** Greene Publishing Associates and Wiley-Interscience,1989.

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Solutions:-

1x TAE 40 mM tris (pH 7.6), 20 mM acetic acid, 1 mM EDTA.
50x (1L) dissolve in 750 ml distilled water:
242 g tris base (FW = 121)
57.1 ml glacial acetic acid
100 ml 0.5 M EDTA (pH 8.0).
Fill to 1 litre with distilled water.

1x TBE 89 mM tris (pH 7.6), 89 mM boric acid, 2 mM EDTA
10x (1L) dissolve in 750 ml distilled water:
108 g tris base (FW = 121)
55 g boric acid (FW = 61.8)
40 ml 0.5 M EDTA (pH 8.0)
Fill to 1 litre with distilled water.

Sample Loading Dye
10x sample buffer stock consists of 50% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanole FF in 1x TAE buffer. Only 1–10 ml of the 10x loading dye should be prepared.

Ethidium Bromide Solution
Add 10 mg of Ethidium Bromide to 1 ml distilled water.

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Combs – MC Denotes Multi Channel Pipette compatible.

PHERO-sub 0710-E		PHERO-sub 1010-E		PHERO-sub 1515-E		PHERO-sub 2020-E	
No. of Samples	**Part Number**	**No. of Samples**	**Part Number**	**No. of Samples**	**Part Number**	**No. of Samples**	**Part Number**
0.75mm Thick Combs							
1	111120-001	**1**	111320-001	**1**	111420-001	**1**	111520-001
2	111120-002	**2**	111320-002	**2**	111420-002	**2**	111520-002
4	111120-003	**4**	111320-003	**4**	111420-003	**4**	111520-003
8	111120-004	**8**	111320-004	**10**	111420-004	**10**	111520-004
8 MC	111120-005	**10 MC**	111320-005	**10 MC**	111420-005	**16**	111520-005
10	111120-006	**12**	111320-006	**12**	111420-006	**20 MC**	111520-006
12	111120-007	**16**	111320-007	**14 MC**	111420-007	**25**	111520-007
16	111120-008	**20 MC**	111320-008	**16**	111420-008	**30**	111520-008
				18 MC	111420-009	**36**	111520-009
				20	111420-010	**40 MC**	111520-010
				28 MC	111420-011		
				30	111420-012		

PHERO-sub 0710-E		PHERO-sub 1010-E		PHERO-sub 1515-E		PHERO-sub 2020-E	
No. of Samples	**Part Number**	**No. of Samples**	**Part Number**	**No. of Samples**	**Part Number**	**No. of Samples**	**Part Number**
1mm Thick Combs							
1	111121-001	**1**	111321-001	**1**	111421-001	**1**	111521-001
2	111121-002	**2**	111321-002	**2**	111421-002	**2**	111521-002
4	111121-003	**4**	111321-003	**4**	111421-003	**4**	111521-003
8	111121-004	**8**	111321-004	**10**	111421-004	**10**	111521-004
8 MC	111121-005	**10 MC**	111321-005	**10 MC**	111421-005	**16**	111521-005
10	111121-006	**12**	111321-006	**12**	111421-006	**20 MC**	111521-006
12	111121-007	**16**	111321-007	**14 MC**	111421-007	**25**	111521-007
16	111121-008	**20 MC**	111321-008	**16**	111421-008	**30**	111521-008
				18 MC	111421-009	**36**	111521-009
				20	111421-010	**40 MC**	111521-010
				28 MC	111421-011		
				30	111421-012		

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PHERO-sub 0710-E		PHERO-sub 1010-E		PHERO-sub 1515-E		PHERO-sub 2020-E	
No. of Samples	**Part Number**	**No. of Samples**	**Part Number**	**No. of Samples**	**Part Number**	**No. of Samples**	**Part Number**
1.5mm Thick Combs							
1	111122-001	**1**	111322-001	**1**	111422-001	**1**	111522-001
2	111122-002	**2**	111322-002	**2**	111422-002	**2**	111522-002
4	111122-003	**4**	111322-003	**4**	111422-003	**4**	111522-003
8	111122-004	**8**	111322-004	**10**	111422-004	**10**	111522-004
8 MC	111122-005	**10 MC**	111322-005	**10 MC**	111422-005	**16**	111522-005
10	111122-006	**12**	111322-006	**12**	111422-006	**20 MC**	111522-006
12	111122-007	**16**	111322-007	**14 MC**	111422-007	**25**	111522-007
16	111122-008	**20 MC**	111322-008	**16**	111422-008	**30**	111522-008
				28 MC	111422-009	**36**	111522-009
				20	111422-010	**40 MC**	111522-010
				28	111422-011		
				30	111422-012		

PHERO-sub 0710-E		PHERO-sub 1010-E		PHERO-sub 1515-E		PHERO-sub 2020-E	
No. of Samples	**Part Number**	**No. of Samples**	**Part Number**	**No. of Samples**	**Part Number**	**No. of Samples**	**Part Number**
2mm Thick Combs							
1	111123-001	**1**	111323-001	**1**	111423-001	**1**	111523-001
2	111123-002	**2**	111323-002	**2**	111423-002	**2**	111523-002
4	111123-003	**4**	111323-003	**4**	111423-003	**4**	111523-003
8	111123-004	**8**	111323-004	**10**	111423-004	**10**	111523-004
8 MC	111123-005	**10 MC**	111323-005	**10 MC**	111423-005	**16**	111523-005
10	111123-006	**12**	111323-006	**12**	111423-006	**20 MC**	111523-006
12	111123-007	**16**	111323-007	**14 MC**	111423-007	**25**	111523-007
16	111123-008	**20 MC**	111323-008	**16**	111423-008	**30**	111523-008
				18 MC	111423-009	**36**	111523-009
				20	111423-010	**40 MC**	111523-010
				28 MC	111423-011		
				30	111423-012		

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Revision: 1.0

PHERO-sub 2632-E Combs:

Description	Part Number
Comb 28 sample MC, 0.75mm thick	111620-001
Comb 56 sample MC, 0.75mm thick	111620-002
Comb 28 sample MC, 1mm thick	111621-001
Comb 56 sample MC, 1mm thick	111621-002
Comb 28 sample MC, 1.5mm thick	111622-001
Comb 56 sample MC, 1.5mm thick	111622-002
Comb 28 sample MC, 2mm thick	111623-001
Comb 56 sample MC, 2mm thick	111623-002

PHERO-sub 1008-E Combs:-

Description	Part Number
Comb 1 sample , 1 dye , 1mm thick	111220-001
Comb 4 sample, 1mm thick	111220-002
Comb 8 sample, 1mm thick	111220-003
Comb 12 sample, 1mm thick	111220-004
Comb 16 sample, 1mm thick	111220-005
Comb 20 sample MC, 1mm thick	111220-006
Comb 1 sample , 1 dye , 1.5mm thick	111221-001
Comb 4 sample, 1.5mm thick	111221-002
Comb 8 sample, 1.5mm thick	111221-003
Comb 12 sample, 1.5mm thick	111221-004
Comb 16 sample, 1.5mm thick	111221-005
Comb 20 sample MC, 1.5mm thick	111221-006

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Warranty

The multiSUB Horizontal Electrophoresis units have a warranty against manufacturing and material faults of twelve months from date of customer receipt.

If any defects occur during this warranty period, your supplier will repair or replace the defective parts free of charge.

This warranty does not cover defects occurring by accident or misuse or defects caused by improper operation.

Units where repair or modification has been performed by anyone other than your supplier or an appointed distributor or representative are no longer under warranty from the time the unit was modified.

Units which have accessories or repaired parts not supplied by your supplier or it's associated distributors have invalidated warranty.

Your supplier cannot repair or replace free of charge units where improper solutions or chemicals have been used. For a list of these please see the Care and Maintenance subsection.

If a problem does occur then please contact your supplier.